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## INCIDENCE OF FUNGI AND AFLATOXIN CONTAMINATION OF MAIZE IN TOLON-KUMBUNGU DISTRICT OF GHANA

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### ABSTRACT

Maize (*Zea mays* L.) is an important staple food crop and a source of income to farmers, as well as foreign exchange earner in most countries in sub-Saharan Africa. Its production is hampered by fungal diseases, which also cause contamination with mycotoxins, especially aflatoxin and its associated health hazards. This study sought to isolate and identify aflatoxigenic fungi, as well as detect the presence of Aflatoxin B<sub>1</sub> (Afb<sub>1</sub>) in maize samples obtained from farmers in the Tolon-Kumbungu district in the northern region of Ghana. Twenty farming communities were randomly selected for the study in consultation with the district office of the Ministry of Food and Agriculture (MoFA). Samples were collected from 200 randomly selected maize farmers by the composite sampling technique, for isolation of aflatoxigenic fungi by the agar plate method and the detection of aflatoxin. Aflatoxin was detected in maize samples with the Black light, rapid screening and immunoassay methods. *Aspergillus flavus* had the highest percentage of occurrence (63.7%); followed by *A. niger* (16.5%), *Rhizopus stolonifer* (9.3%), *Penicillium* spp. (6.9%) and *Fusarium oxysporum* (3.7%). Farm samples had more aflatoxin than those from stores and markets. Samples of maize from farms in Gbirimani community had the highest aflatoxin contamination of +60 ppb. Concentrations of Afb<sub>1</sub> at or above +20 ppb were recorded in all the communities, except in Tinguli. Apart from Voggu, all market samples were free from aflatoxin contamination.

**Key Words:** Aflatoxigenic fungi, postharvest, *Zea mays*

### RÉSUMÉ

Le maïs (*Zea mays* L.) est une importante culture vivrière de base et une source de revenus pour les agriculteurs, ainsi que des sources de devises dans la plupart des pays Afrique sub-saharienne. Sa production est entravée par des maladies fongiques, qui provoquent également une contamination par des mycotoxines, en particulier l'aflatoxine et les risques pour la santé qui y sont associés. Cette étude visait à isoler et à identifier les champignons aflatoxigènes, ainsi qu'à détecter la présence

d'aflatoxine B1 (AfB1) dans des échantillons de maïs provenant d'agriculteurs du district de Tolon-Kumbungu dans la région nord du Ghana. Vingt communautés agricoles ont été sélectionnées au hasard pour l'étude en consultation avec le bureau de district du ministère de l'Alimentation et de l'Agriculture (MoFA). Des échantillons ont été prélevés auprès de 200 producteurs de maïs sélectionnés au hasard par la technique d'échantillonnage composite, pour l'isolement des champignons aflatoxigènes par la méthode de la plaque de gélose et la détection de l'aflatoxine. L'aflatoxine a été détectée dans des échantillons de maïs avec la lumière noire, des méthodes de dépistage rapide et d'immunodosage. *Aspergillus flavus* avait le pourcentage d'occurrence le plus élevé (63,7%); suivi par *A. niger* (16,5%), *Rhizopus stolonifer* (9,3%), *Penicillium* spp. (6,9%) et *Fusarium oxysporum* (3,7%). Les échantillons de ferme contenaient plus d'aflatoxine que ceux des magasins et des marchés. Les échantillons de maïs provenant d'exploitations agricoles de la communauté de Gbirimani présentaient la contamination d'aflatoxine la plus élevée, soit +60 ppb. Des concentrations d'AfB1 égales ou supérieures à +20 ppb ont été enregistrées dans toutes les communautés, sauf à Tinguli. À l'exception de Voggu, tous les échantillons du marché étaient exempts de contamination par les aflatoxines.

*Mots Clés:* Champignons aflatoxigènes, post-récolte, *Zea mays*

## INTRODUCTION

Maize (*Zea mays* L.), a cereal crop, is grown throughout the world, in a range of agro-ecological zones. According to the IITA (2009), more maize is produced annually than any other grain. Global maize production increased from 200 to 600 million tonnes from 1963 to 2003 (FAOSTAT, 2006) and reached 800 million tonnes in 2012 (FAOSTAT, 2012). Maize forms an important component of the diets of many people in the world, especially in developing countries. In Ghana, it is a staple, which is eaten in various forms such as Tuo Zafi (TZ), kenkey, banku and porridge. Its cultivation is also a source of income and foreign exchange for the country.

Cultivation of maize is hampered by diseases, which are mostly caused by fungi, with *Aspergillus flavus* being an important postharvest fungus in grain stores. Among mycotoxins, aflatoxin produced by *A. flavus* is the most common (Sowley, 2016). Farmers' preharvest, harvest and postharvest activities, and other factors such as temperature, humidity and pest damage affect the level of infection of grains by mycotoxigenic fungi. Fungal infection and/or contamination of foodstuff, animal feed and crops with toxic metabolites results in huge losses (FAO,

2005). Mycotoxins contaminate maize in the field and during storage, thus making the grains unwholesome for consumption. The predisposing factors of infection include improper drying, high relative humidity and temperature, farmers' production practices such as intercropping with aflatoxin-contaminated grains, early and delayed, harvesting and poorly constructed storage structures (Sowley, 2016).

In spite of the huge losses and harmful effects caused by fungi or their metabolites, most farmers remain oblivious. A number of studies have revealed the prevalence of fungi and their metabolites, especially the aflatoxins. According to Ayalew (2010), maize a staple in developing countries can easily be contaminated with aflatoxins. He detected aflatoxin in 88% of maize samples, with concentrations below 5  $\mu\text{g kg}^{-1}$ ; except in one sample which had 27  $\mu\text{g kg}^{-1}$ . Bankole and Adebajo (2003) detected aflatoxin B<sub>1</sub> level up to 14 g kg<sup>-1</sup> and aflatoxin G<sub>1</sub> level up to 58 g kg<sup>-1</sup> in stored maize in Benin. Agbetiamah (2018) reported that 35% of 509 (326 maize and 183 groundnut) samples had detectable aflatoxin. Over 15% of the maize sample exceeded the aflatoxin threshold limit of 15 ppb set by the Ghana Standards Authority. Sekiyama (2005) also detected aflatoxins in

3.2% of maize-based food samples in Brazil. Contamination of maize with fungi and their metabolites such as mycotoxins has resulted in low patronage of maize in Northern Ghana (Nestlé, 2010). According to Sowley (2016), the prevalence of mycotoxins, especially aflatoxins and its associated health problems is a silent threat in developing countries where most of the people are oblivious of the dangers associated with the consumption of contaminated produce.

The objective of the study was to isolate and identify aflatoxigenic fungi and detect aflatoxin in maize grain samples from farms, stores and markets in Ghana.

## MATERIALS AND METHODS

**Study area.** The study was conducted in the Tolon-Kumbungu district of the Northern Region, which lies between latitudes 10 and 20°N and longitudes 10 and 50°W. The district covers an area of about 2,741 Km<sup>2</sup>, and is one of the largest cereal-growing districts in the Northern Region.

With the assistance of the Ministry of Food and Agriculture (MoFA) District Office, 20 communities, namely Dasuyili, Gbirimani, Tinguli, Dimabe, Gawagu, Sabegu, Dundo, Yoggu, Waribogu, Tali, Gbullung, Gbirimanchang, Kumbungu, Kumbungu-Yipelgu, Chirifoyili, Lungbun, Dalun, Kasuyili, Voggu and Zali were selected for the study.

### Sampling

Maize samples were collected from randomly selected farmers in eight out of the 20 communities. The samples were collected from two farms, two storage facilities and one market in each of the eight communities. In the field, 30 cobs were collected for every 0.5 ha; but where the farmers had already shelled their harvest, a minimum of 1 kg of grain was collected. Where grains were stored in sacks, five perimeter samples and one centre sample were collected using a probe and combined to obtain a composite sample of at least 5 kg.

Three sub-samples weighing 1 kg each were obtained from shelled maize, using the

coning and quartering method, to ensure good mixing and representation. One of the sub-samples was used for physical assessment of aflatoxin contamination using UV light. The other was used for fungal isolation and aflatoxin measurement while the third was stored as a retained sample.

### Isolation and identification of fungi

**Media preparation.** Potato Dextrose Agar (PDA) (Oxoid Ltd. Basingstoke, UK) was prepared according to the manufacturer's instructions of 39 g of the dry powder per litre of water. The mouth of the flask containing the PDA and water mixture was plugged with cotton wool and covered with aluminium foil before sterilising in an autoclave at a temperature of 121 °C and a pressure of 1.05 kg cm<sup>2</sup> for 15 minutes.

**Isolation.** Prior to plating, maize grains were surface sterilised for five minutes, with 10% sodium hypochlorite and rinsed in sterile distilled water. Isolations were made from two equal lots of sterilised and unsterilised grain by plating on PDA plates. The plates were incubated under alternating cycles of 12 hr light and darkness, at room temperature; and examined after seven days. Cultural characteristics of fungi which grew from the grains were observed and recorded to aid in identification.

**Identification of fungi.** Fungi isolated from the grains were identified on the basis of colony and spore characteristics such as shape, size and colour with the aid of a stereomicroscope and a compound microscope. An illustrated manual on identification of seed-borne *Aspergillus*, *Fusarium*, *Penicillium* species and their mycotoxins was used (Bhadauria and Gautam, 2018).

**Occurrence of fungi.** The percentage occurrence of fungi was determined as follows:

Percentage occurrence =  $n/N \times 100$   
 ..... Equation 1

Where:

$n$  = number of times a fungus occurred  
 $N$  = total number of fungi per plate

**Black light test.** This is a quick preliminary test that involved a visual inspection for the presence of a greenish-gold fluorescence under light at a wavelength of 365 nm. A 2 kg sample of grain from each of the sites, was discharged in a monolayer onto a black tray in an opaque box. The grains were then observed for the presence of a greenish-gold fluorescence under light at a wavelength of 365 nm. Where more than four glowing grain particles per 2 kg sample were observed, the aflatoxin contamination was likely to be above 20 ppb.

**Rapid screening test.** The Quicking Aflatoxin B<sub>1</sub> Precise test kit (Quicking Biotech Co., Ltd., China) was used to detect the presence and level of aflatoxin in each sample. It is a competitive immunoassay for the semi-quantitative detection of the presence of aflatoxin B<sub>1</sub>. A 20 ppb cut-off level was chosen in order to determine if the grains were within acceptable safe range for human and livestock consumption. Allowable assay time was 5 - 10 minutes.

The rapid screening test was performed with coarsely ground samples weighing 25 g. To determine the level of aflatoxin at 20 ppb, 2 g of the ground portion was weighed with an electronic balance, and mixed with 2 ml of distilled water and 8 ml of ethyl acetate before centrifuging at 4000 rpm for one minute. Two millilitres of the supernatant (ethyl acetate layer) was then collected into a small tube using a microlitre pipette and dried by blowing. The residue was re-dissolved in 1.6 ml of assay diluents to obtain a safe cut-off level of 20 ppb.

**Competitive immunoassay detection of the presence of aflatoxin B<sub>1</sub> (afb<sub>1</sub>).** Five drops

of the diluted extract above was passed through a micro-well provided in the test kit and the results recorded after 5 min, using the key provided below:

Positive test result (aflatoxin present at or above 20 ppb cut-off level): One clear band in C zone of the cassette indicated a positive result, which implied that concentration of Afb<sub>1</sub> was at or above 20 ppb in the sample.

Negative test result (no aflatoxin present at 20 ppb cut-off level): The presence of bands C and T indicated a negative result.

Invalid: No band appeared in C zone.

## RESULTS

**Percentage occurrence of fungi.** Five fungal species, namely *Aspergillus flavus*, *A. niger*, *Rhizopus stolonifer*, *Fusarium oxysporum* and *Penicillium* spp., were isolated. *Aspergillus flavus* had the highest occurrence (63.7%) followed; by *A. niger* (16.5), *R. stolonifer* (9.3), *Penicillium* spp. (6.9) and *F. oxysporum* (3.7%) (Fig. 1). All the fungi isolated in this study are aflatoxigenic, except *Rhizopus stolonifer*. *Aspergillus flavus* was the most common fungus associated with the maize samples.

### Detection of aflatoxins

**Black light test.** Generally, farm samples had more aflatoxin than those from stores and markets (Table 1). Apart from Dimabe, aflatoxin was detected in samples from the rest of the communities, with Gbirmani recording the highest in the farm (60 ppb) and in the stores (20 ppb). Among market samples, aflatoxin was detected only in those collected from Voggu. For samples collected from stores, aflatoxin was detected only in those collected from Gbirmani. Total amounts of aflatoxin in farm, market and store samples were 250 ppb, 40 ppb and 20 ppb, respectively. The sign test revealed that grains collected from markets had a lower probability ( $P=0.0352$ ) of contamination by aflatoxin B<sub>1</sub> at 20 ppb cut-off level; while grains from

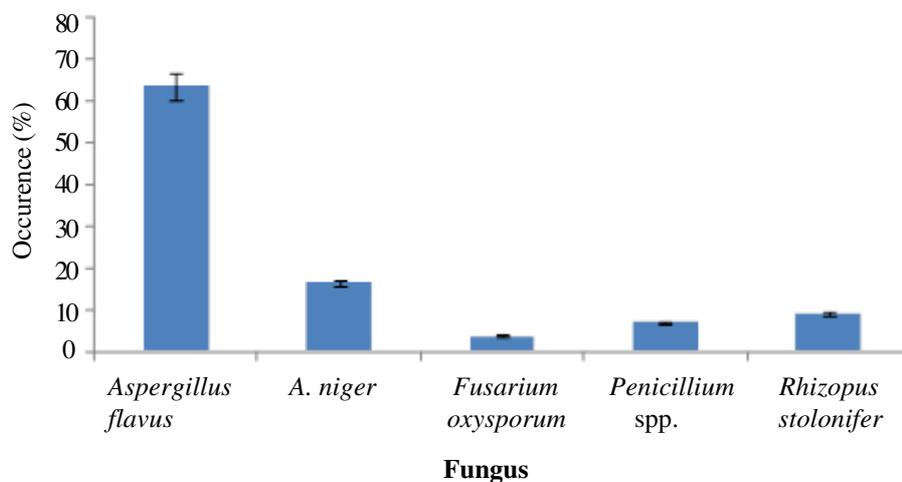


Figure 1. Fungi isolated from maize grains samples collected from farms, markets and stores in Tolon-Kumbungu district in Ghana.

TABLE 1. Levels of aflatoxin detected in farm, store and market maize grain samples in Tolon-Kumbungu district of Ghana

Community	Aflatoxin level in ppb		
	Farm	Store	Market
Tinguli	+20	0	0
Gawagu	+30	0	0
Sabegu	+35	0	0
Dimabe	0	0	0
Gbirmani	+60	+20	0
Voggu	+50	0	+40
Waribogu	+35	0	0
Kumbungu-Yipelgu	+20	0	0
Total	+250	+20	+40

farms ( $P=0.3633$ ) and stores ( $P=0.3633$ ) were more likely to be contaminated by aflatoxin  $B_1$  at 20 ppb cut-off level.

**Rapid screening test.** Maize samples collected from farms in Sabegu, Dimabe, Gbirmani, Woribogu and Kumbungu-Yipelgu tested positive for aflatoxin  $B_1$  contamination at 20 ppb cut-off level (Table 2). For store samples,

only those from Gawagu, Sabegu, Dimabe, Gbirmani and Woribogu tested positive. Maize samples collected from the markets were all free from aflatoxin contamination, except those from Voggu which tested positive (Table 2). Farm, store and market samples from Tinguli were all free of aflatoxin.

TABLE 2. Presence or absence of Aflatoxin B<sub>1</sub> at 20 ppb cut-off in maize grain samples collected from farms, stores and markets in Tolon-Kumbungu district in Ghana

Community	Presence or absence of aflatoxin		
	Farm	Store	Market
Tinguli	-	-	-
Gawagu	-	+	-
Sabegu	+	+	-
Dimabe	+	+	-
Gbirimani	+	+	-
Voggu	-	-	+
Waribogu	+	+	-
Kumbungu-Yipelgu	+	-	-

+ = Aflatoxin B<sub>1</sub> present at 20 ppb cut-off level, - = Aflatoxin B<sub>1</sub> absent at 20ppb cut-off level

## DISCUSSION

### Occurrence of seed-borne fungi.

*Aspergillus flavus*, *A. niger*, *Fusarium oxysporum*, *Penicillium* spp. and *Rhizopus stolonifer* were isolated, with *A. flavus* as the most common (63.72%). Among the five fungi, only *R. stolonifer* is not a known aflatoxigenic fungus. Muthomi *et al.* (2009) isolated fungi from the genera *Aspergillus*, *Fusarium* and *Penicillium* with *Fusarium* as the most frequent and *Aspergillus niger* and *A. flavus* having a higher frequency among the *Aspergilli*. Enyisi *et al.* (2015) also isolated fungi belonging to the genera *Aspergillus*, *Penicillium*, *Rhizopus*, *Fusarium* and *Botrytis* with *Aspergillus* as the most predominant (62%) genus.

The high occurrence of *A. flavus* in maize could have resulted from farmers' practice of allowing grains to dry out on the farm thereby, exposing them to mould infection. Rachaputi *et al.* (2002) reported that field fungi, such as *Fusarium* spp., contaminate grains before or during harvest. According to Muthomi *et al.* (2009), poor harvesting practices, improper storage and less than optimal conditions during transportation, marketing and processing can also contribute to fungal growth and increase in the risk of mycotoxin production.

Storage fungi such as *Penicillium* spp. (6.8%) and *Aspergillus niger* (16.5%) are reported to require much lower moisture than the field fungi and, therefore, tend to contaminate grain in silos and other storage places (Rachaputi *et al.*, 2002). It is known that aflatoxin production is favoured by prolonged drought and associated elevated temperatures; but Choudhary *et al.* (2005) reported that some of the storage fungi (responsible for post-harvest mycotoxin production) get associated with food grain from the field (pre-harvest stage). It is, therefore, possible that *A. niger* and *Penicillium* spp. were carried in contaminated grain from the field to storage through contact with soil at harvest.

### Detection and quantification of aflatoxins

**Black light test.** The black light test showed that aflatoxins were present in seven of the eight farm samples tested (Table 1). This was confirmed by the occurrence of greenish-gold fluorescence among the samples. Contamination levels recorded for samples from Gbirimani (60 ppb) and Voggu (50 ppb) could be attributed to poor compliance with Good Agricultural Practices in the field by farmers, which can significantly reduce or

prevent mycotoxin contamination (Sowley, 2016). Aflatoxin contamination of maize grain from stores and markets was generally lower than that from farms. The highest level of contamination was recorded in grain samples collected from a market in Voggu (40 ppb). Records of less than four glowing particles per 2 kg sample and in most cases, no greenish gold fluorescence, were observed. This could have resulted from a combination of good farmers' knowledge about storage and good storage practices exhibited by the farmers (Choudhary *et al.*, 2005).

**Rapid screening test.** Presence of aflatoxin B<sub>1</sub> at 20 ppb cut-off level was estimated in grain obtained from farms, stores and markets. Field samples from five out of the eight communities were positive for aflatoxin B<sub>1</sub> at or higher than 20 ppb (Table 2). This was confirmed by the presence of only one clear band that appeared in the C zone of the test kit. Exposure of plants to drought stress during seed development and maturation (98%), especially in tropical conditions could have also contributed to the contamination. Gnonlonfin *et al.* (2013) reported that mycotoxin contamination is a generally climate-dependent, plant and storage-associated problem, associated with many factors (e.g., biological factors, harvesting, storage and processing conditions, moisture content), insect damage, and pre and post-harvest handling.

Store samples collected from five out of the eight communities tested positive to aflatoxin B<sub>1</sub> contamination at 20 ppb cut-off level. Contamination of stored grains could have originated from the farm, as reported by Choudhary *et al.* (2005) who indicated that post-harvest contamination is usually the result of pre-harvest presence of fungal contamination.

Prevalence of aflatoxin in most farm and store samples confirms reports by Karthikeyan *et al.* (2013) and Ahsan *et al.* (2010) that most of their samples had aflatoxin levels above the acceptable limit of 20 µg kg<sup>-1</sup>. Variation in aflatoxin concentration among samples could

be due to differences in moisture content, period of storage or nature of storage. Market samples were relatively free of aflatoxin B<sub>1</sub> contamination at 20 ppb, except that from Voggu. This could have resulted from the proper drying, rigorous cleaning and sorting. Hamilton (2000) confirmed that drying harvested maize to 15.5% moisture content or lower within 24 - 48 hrs of harvest will reduce the risk of fungal growth and aflatoxin production.

## CONCLUSION

Five fungi namely *Aspergillus flavus*, *A. niger*, *Penicillium* spp., *Fusarium oxysporum* and *Rhizopus stolonifer* were isolated from maize grain samples obtained from farms, stores and markets in Tolon-Kumbungu district in Ghana. Out of these, only *R. stolonifer* is not a known mycotoxigenic fungus. *Aspergillus flavus* which produces aflatoxin was the most common fungus associated with the maize samples.

Field samples had relatively more aflatoxin than those from stores. All market samples were free of aflatoxin, except those from Voggu. Farmers need to adopt good agricultural practices in to minimise or eliminate grain contamination.

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